

## PATENT ABSTRACTS OF JAPAN

(11)Publication number : 06-298695

(43)Date of publication of application : 25.10.1994

(51)Int.Cl.

C07C 49/577

A01N 35/04

(21)Application number : 05-109984

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(22)Date of filing : 12.04.1993

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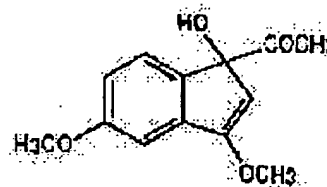
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**(54) INDENE DERIVATIVE AND ANTIBACTERIAL AGENT CONTAINING THE DERIVATIVE AS ACTIVE COMPONENT****(57)Abstract:**

**PURPOSE:** To obtain a new indene derivative having excellent antimicrobial activity on bacteria and usable as an antibacterial agent for foods, cosmetics, etc., by extracting ground adlay sprout, freeze-dried adlay sprout, etc., with a lower alcohol.

**CONSTITUTION:** Seed of adlay of TOKUDA variety is cultured in a dark place at 25°C for 4 days to obtain adlay sprout. The sprout is ground and freeze-dried. The freeze-dried product is extracted with methyl alcohol, the extraction mixture is filtered to separate the extract liquid and the liquid is concentrated under reduced pressure to obtain an extract. Methyl alcohol and chloroform are added to the extract and mixed by stirring, a 0.8% aqueous solution of potassium chloride is added thereto, the mixture is left to stand and the chloroform layer is separated and concentrated under reduced pressure. The residue is extracted with acetone, the extract is fractionated by gel-permeation chromatography, high-performance liquid chromatography, etc., and a fraction



having high antibacterial activity is recovered to obtain the objective indene derivative expressed by formula and having excellent antibacterial activity.

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## LEGAL STATUS

[Date of request for examination] 15.07.1996

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number] 2754313

[Date of registration] 06.03.1998

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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## DETAILED DESCRIPTION

## [Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the antimicrobial agent which makes an indene derivative and this an active principle.

[0002]

[Description of the Prior Art] It is known that Coix lacryma-joli also has antimicrobial activity in the dry matter, the juice liquid, or the extract of a coconut, and the monoglyceride of stearin acid or a palmitic acid or these derivatives are suggested as a compound in which this antimicrobial activity is shown (JP, 2-270825, A, JP, 3-240473, A).

[0003]

[Problem(s) to be Solved by the Invention] It is isolated and this invention offers [ Coix lacryma-joli mist deer ] the new indene derivative in which the outstanding antimicrobial activity is shown.

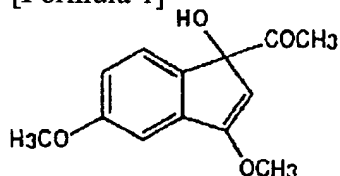
[0004]

[Means for Solving the Problem] a deer -- carrying out -- this invention persons -- Coix lacryma-joli -- the result wholeheartedly studied about the antimicrobial activity which the dry matter, the juice liquid, or the extract of bean sprouts shows -- Coix lacryma-joli -- when predetermined processing was performed to bean sprouts, the new indene derivative was isolated and it found out that the antimicrobial activity excellent in this indene derivative was shown.

[0005] That is, this invention relates to the antimicrobial agent which makes an active principle the indene derivative and this which are shown by the following formula 1.

[0006]

[Formula 1]



[0007] the indene derivative shown by the formula 1 -- Coix lacryma-joli -- it is isolated by processing bean sprouts as follows. first, it mentions later in the example in detail -- as -- the Coix lacryma-joli seed -- dark culture -- carrying out -- Coix lacryma-joli -- bean sprouts are obtained. The target Coix lacryma-joli seed does not have especially a limit in the forms, such as the Tokuda native species, the Nakazato native species, the Okayama native species, and the Kuroishi native species. Moreover, that general in dark culture can also follow a coconut dark culture condition. For example, what is necessary is just to carry out grade dark culture of the Coix lacryma-joli seed for four - eight days at 25 degrees C. Coix lacryma-joli obtained in this way -- bean sprouts can use at least the all.

[0008] next, this is also later mentioned in the example in detail -- as -- above-mentioned Coix lacryma-joli -- bean sprouts, its grinding object, and a freeze-drying object -- extract processing of the grinding

object etc. is further carried out with lower alcohol, such as methyl alcohol and ethyl alcohol, liquid-liquid distribution extract processing of the extract is carried out with chloroform, an acetone, etc., and acetone soluble simple lipid is obtained.

[0009] Finally, the above-mentioned simple lipid is repeated, chromatography fractionation processing is carried out and a desired compound is isolated so that this may also be later mentioned in the example in detail. As for chromatography fractionation processing, it is desirable to carry out combining gel permeation chromatography and high performance chromatography using the mobile phase from which a polarity differs.

[0010] The structural-analysis result of the compound isolated in this way is as follows.

(1) Molecular weight : 234 (C<sub>13</sub>H<sub>14</sub>O<sub>4</sub>)

(2) Infrared absorption spectrum : 1569, 1712, 3401 cm<sup>-1</sup> (3) nuclear-magnetic-resonance spectrum (1 H-NMR, delta): 2.01 (3H, s), 3.84 (3H, s), 4.00 (3H, s), 5.68 (1H, s), 7.08 (5H, dd, J=8.2, 6.7), 7.37 (1H, d, J=2.67), 7.57 (1H, d, J=8.55)

(4) Nuclear-magnetic-resonance spectrum (13 C-NMR, delta) : 31.6, 55.9, 57.1, 76.0, 99.1, 109.5, 117.2, 128.7, 129.0, 137.0, 160.3, 168.3, 205.0

[0011] The compound isolated from the above-mentioned structural-analysis result is an indene derivative shown by the formula 1, and is 1-acetyl-1-hydroxy-3,5-dimethoxy-1H-indene was determined.

[0012] The indene derivative shown by the formula 1 shows the antimicrobial activity which was excellent to bacteria, and the use to food, cosmetics, etc. attracts attention as a natural antimicrobial agent so that it may mention later in the example in detail.

[0013]

[Example]

Test partition 1 (isolation of an indene derivative)

the Coix lacryma-joli seed of the Tokuda native species which gathered a harvest -- 25 degrees C -- the dark culture during four days -- carrying out -- Coix lacryma-joli -- bean sprouts -- obtaining -- this Coix lacryma-joli -- after grinding bean sprouts, it freeze-dried at the shelf temperature of 20 degrees C, and the freeze-drying object was obtained.

[0014] It filtered and the extract was obtained, after having added 6l. of methyl alcohol to the 150g of the above-mentioned freeze-drying objects, carrying out homogenization processing and leaving this at a room temperature for 48 hours. 6l. of methyl alcohol was added to residue, extract processing was performed similarly, the extract was obtained, and this was doubled with the 1st extract. And 30g of extracts was obtained by heating at 40-45 degrees C, and evaporating methyl alcohol under reduced pressure of the doubled extract.

[0015] Methyl alcohol 100ml and chloroform 200ml were added to the 30g of the above-mentioned extracts, mixed churning was carried out, and 60ml of 0.8 more% potassium chloride water solutions was added and put. The chloroform layer was isolated preparatively, after heating at 40-45 degrees C under reduced pressure and condensing to 1ml, 0.2ml of methyl alcohol nature magnesium chloride solutions was added 10% with acetone 10ml, mixed churning was carried out, after 1-hour ice-cooling, centrifugal separation was carried out and the acetone solution of supernatant liquid was isolated preparatively. 0.2ml of methyl alcohol nature magnesium chloride solutions was added to residue 10% with acetone 10ml, it processed similarly, the acetone solution was obtained, and this was doubled with the 1st acetone solution. And 7.1g of acetone soluble simple lipid was separated by heating at 40-45 degrees C, and evaporating an acetone under reduced pressure of the set acetone solution.

[0016] The 7.1g of the above-mentioned simple lipid is dissolved under chloroform, and it is JAIGERU. Two gel permeation chromatography was performed using 1H (JAIGEL 1H, a trade name, Japanese analysis industrial company make, 8.0mm phix500mm). Under the present circumstances, it was made to flow down this chloroform by part for 3.5ml/of the rates of flow, using chloroform as a mobile phase. It detected by RI (refractive index) and four fractions which show main peaks were obtained. Bacillus Subtilis, Saccharomyces SEREBISHIE, Aspergillus The antibacterial trial of each fraction was performed with the paper disk method by having made nigre into the assay strain, and 1.8g of fractions for Rt(it is the same holding-time and the following) = 55 - 64 minutes which show the strongest

antimicrobial activity was obtained.

[0017] The 1.8g of the above-mentioned fractions is dissolved with methyl alcohol, and it is a cap cel. High performance chromatography was performed using the pack C18 (it is the same CAPCELL PAK C [18], a trade name, the Shiseido Co., Ltd. make, 20mmmm [phix250], and the following). Under the present circumstances, it left by methyl alcohol / water = 20 / 80 (weight ratio) as a mobile phase, the straight-line gradient which becomes 100/0 (weight ratio) after 30 minutes was performed, and it was made to flow down this mobile phase by part for 3.0ml/of the rates of flow. It detected by UV (it is the same an ultraviolet-rays absorbance, 254nm, and the following), and six fractions which show main peaks were obtained. By the same antibacterial trial as the above, 146.4mg of fractions for  $R_t = 34 - 38$  minutes which show the strongest antimicrobial activity was obtained.

[0018] The 146.4mg of the above-mentioned fractions is dissolved with methyl alcohol, and it is a cap cel. High performance chromatography was performed using the pack C18. Under the present circumstances, it was made to flow down this mobile phase by part for 3.0ml/of the rates of flow, using methyl alcohol / water = 50 / 50 (weight ratio) as a mobile phase. It detected by UV and eight fractions which show main peaks were obtained. By the same antibacterial trial as the above, it is a bacillus among assay strains. About the fraction for  $R_t = 30$  minutes which shows antimicrobial activity only to subtilis, it is a cap cel again. Pack High performance chromatography was performed using C18. Under the present circumstances, it was made to flow down this mobile phase by part for 3.0ml/of the rates of flow, using methyl alcohol / water = 25 / 75 (weight ratio) as a mobile phase. It detected by UV and 1.5mg of compounds for  $R_t = 23$  minutes was isolated.

[0019] It is 1-acetyl-1-hydroxy [ the structural-analysis result of the compound isolated in this way is as having mentioned above, and this compound is indicated to be by the formula 1 ]. - It was a 3 and 5-dimethoxy-1H-indene.

[0020] Test partition 2 (antimicrobial activity of an indene derivative)

Antimicrobial activity is a bacillus. It examined with the paper disk method by making subtilis into an assay strain. Under the present circumstances, the following tryptosoy agar (1.5% of agars) was used, impregnation solidification of the 15ml of these agar media was carried out at the petri dish of 90mm of diameters, and plate agar was produced.

Tryptosoy agar (NISSUI PHARMACEUTICAL CO., LTD. make)

Peptone 1.5% and soybean peptone 0.5% and NaCl 0.5% and 1.5% of agars [0021] The assay strain was inoculated into 15ml of the same agar media as the above, and multistory [ of this ] was carried out on the above-mentioned plate agar, and it solidified. The paper disc (thin [ Toyo Roshi and 8mm of diameters ]) was placed on the plate agar which carried out multistory, 20micro (10mg/(ml)) of compounds isolated to this paper disc was dropped 1 times (200microg / paper disc), and the growth inhibition diameter of circle was measured after 24-hour culture. The average of five paper discs was taken and it asked for antimicrobial activity by the following formula 2. Antimicrobial activity is 24.0mm and it was authorized that the isolated compound shows the antimicrobial activity which was excellent to bacteria.

[Formula 2] The diameter of an antimicrobial activity (mm) = inhibition diameter-of-circle (mm)-paper disc (8mm)

[0022]

[Effect of the Invention] There is already effectiveness that it is effective as an antimicrobial agent in the indene derivative of this invention explained above so that clearly.

[Translation done.]